

1. (Original) A photoreactive azido-ruthenium (AzRu) compound which binds to a  $\text{Ca}^{2+}$ -binding protein.
2. (Original) The compound of claim 1, wherein the molar ratio between ruthenium and azide in said compound is 2:1.
3. (Previously Presented) The compound of claim 2, comprising in its molecule ruthenium, azido group, and chlorine.
4. (Original) The compound of claim 3, wherein the molar ratio  $\text{Ru}:\text{N}_3:\text{Cl}$  is 2:1:5.
5. (Previously Presented) The compound of claim 4, further comprising bound water molecules.
6. (Previously Presented) The compound of claim 5, in which one of the atoms is a radioactive isotope.
7. (Original) The compound of claim 6, wherein the isotope is  $^{103}\text{Ru}$ .
8. (Previously Presented) The compound of claim 1, which compound covalently binds to said  $\text{Ca}^{2+}$ -binding protein following photo-activation by UV irradiation.
9. (Original) The compound of claim 8, which binds to the  $\text{Ca}^{2+}$ -binding site of said  $\text{Ca}^{2+}$ -binding protein.
10. (Previously Presented) The compound of claim 1, which specifically binds to said  $\text{Ca}^{2+}$ -binding protein, thereby inhibiting its  $\text{Ca}^{2+}$ -dependent activity.
11. (Previously Presented) The compound of claim 1, wherein said  $\text{Ca}^{2+}$ -binding protein is selected from the group consisting of proteins involved in signal transduction, muscle contraction, neurotransmitter release, hormone secretion, cell motility,

apoptosis, fertilization, cell proliferation, cell mitosis and in gene expression; proteins associated with  $\text{Ca}^{2+}$ -transport,  $\text{Ca}^{2+}$ -pumps, and with the mitochondrial uniporter; channel protein VDAC;  $\text{Ca}^{2+}$ -release channel/ryanodine receptor;  $\text{IP}_3$  receptor, proteins involved in  $\text{Ca}^{2+}$ -efflux in mitochondria; and soluble  $\text{Ca}^{2+}$  binding proteins regulating various cellular activities.

12. (Previously Presented) The compound of claim 10, wherein the inhibition of the  $\text{Ca}^{2+}$ -dependent activity of said  $\text{Ca}^{2+}$ -binding protein increases by photo-activation, compared to the inhibition without photo-activation.
13. (Previously Presented) A method of isolating a  $\text{Ca}^{2+}$ -binding protein from a source comprising the same, which method comprises the steps of:
  - i) providing a source comprising a  $\text{Ca}^{2+}$ -binding protein;
  - ii) reacting said source with an AzRu compound of claim 1, optionally under photo-activation by UV irradiation, whereby said  $\text{Ca}^{2+}$ -binding protein is bound to said compound;
  - iii) isolating the material bound to said compound obtained in step (ii); and
  - iv) releasing the  $\text{Ca}^{2+}$ -binding protein from the product obtained in step (iii).
14. (Original) A method according to claim 13, wherein the  $\text{Ca}^{2+}$ -binding protein is isolated by affinity chromatography.
15. (Original) A method according to claim 14, wherein said AzRu compound is bound to particles of porous polymer that are packed in a column, and wherein any  $\text{Ca}^{2+}$ -binding protein is retained in said column, while other proteins are eluted.
16. (Previously Presented) A method according to claim 15, wherein said retained  $\text{Ca}^{2+}$ -binding proteins are released from the column by calcium ions.

17. (Previously Presented) A method according to claim 15, wherein said particles comprise agarose, cellulose, or dextran.
18. (Previously Presented) A method for characterizing the structure of a  $\text{Ca}^{2+}$ -binding protein, wherein said  $\text{Ca}^{2+}$ -binding protein has been isolated by a method according to claim 13, further comprising a method selected from the group consisting of electrophoresis, autoradiography, liquid chromatography, MALDI-TOF analysis, LC-MS/MS, protein sequencing, and a sequence homology search.
19. (Original) A method for the preparation of an AzRu-comprising bio-sensor chip comprising the steps of:
  - i) providing an azido-ruthenium compound comprising in its molecule ruthenium, azido group, and chlorine at a molar ratio of 2:1:5;
  - ii) binding said compound to a polymer such as dextran and coupling it to a suitable support, preferably gold-plated surface to give a chip; and
  - iii) optionally stabilizing the resulting chip.
20. (Previously Presented) Use of the chip of claim 19 for the isolation of  $\text{Ca}^{2+}$ -binding proteins, comprising :
  - i) exposing said chip to a biological sample comprising a  $\text{Ca}^{2+}$ -binding protein for a time sufficient for binding of the protein with the support-bound ruthenium compound to occur; and
  - ii) washing said chip with a buffer comprising either calcium or EGTA.
21. (Previously Presented) Use of the chip of claim 20, comprising surface plasmon resonance.
22. (Previously Presented) A method of screening for a calcium-binding substances, preferably  $\text{Ca}^{2+}$ -binding proteins, comprising the steps of:
  - i) providing test substances, preferably proteins;
  - ii) contacting said substances with the ruthenium compound of claim 1 under conditions which allow binding to occur, preferably under UV irradiation;
  - iii) isolating from the reaction of (ii) those substances that specifically bind to said ruthenium compound; and

- iv) releasing the substances obtained in step (iii) from the ruthenium compound by suitable means.
23. (Previously Presented) The method of claim 22 further comprising the step of testing the substances obtained in step (iv) for their  $\text{Ca}^{2+}$ -dependent activity.
24. (Previously Presented) A method according to claim 13, wherein said AzRu compound is labeled.
25. (Previously Presented) A method according to claim 24, wherein said labeling is radioactive labeling.
26. (Previously Presented) A process for preparing a photoreactive azido-ruthenium compound which binds to a  $\text{Ca}^{2+}$ -binding protein, wherein the molar ratio between ruthenium and azide in said compound is 2:1, comprising:
- i) reacting in the dark sodium azide with ruthenium (III) chloride in the presence of HCl;
  - ii) applying the reaction mixture of the previous step onto a chromatographic column selected from cation-exchanger or hydrophobic;
  - iii) collecting the fractions having high absorbance at 290 nm; and optionally
  - iv) drying the collected fractions, redissolving them, rechromatographing them, and optionally crystallizing said compound from methanol.
27. (Previously Presented) The process of claim 26, wherein the HCl has the concentration in the range of from 0.5 mol/l to 2 mol/l.
28. (Previously Presented) The process of claim 26, wherein said sodium azide and ruthenium chloride are reacted at about 100°C for about 2 to 4 hrs.
29. (Previously Presented) The process of claim 26, wherein the product of AzRu migrates as a single spot with  $R_f$  being about 0.9 during TLC on cellulose F plates, using 0.16 M ammonium formate, pH 8.5 and 20% methanol as the developer.

30. (Previously Presented) The process of claim 26, wherein said product is soluble in water, DMF and DMSO, less soluble in methanol, and insoluble in ethanol, ether, chloroform, ethyl acetate, n-butanol, and isopropyl alcohol.
31. (Previously Presented) The process of claim 26, wherein said product has an absorbance maximum at about 290 nm.
32. (Previously Presented) The process of claim 26, wherein said product has molar absorbance of about 15,000 at 290 nm in a water solution.
33. (Previously Presented) Use of an AzRu compound of claim 1 in diagnosing a disorder associated with a defect in the function of a  $\text{Ca}^{2+}$ -binding protein in a subject, comprising:
- i) providing a sample of said subject and a control sample of a normal subject;
  - ii) contacting said samples with an azido-ruthenium compound of claim 1 under conditions suitable for binding to occur, preferably under UV irradiation;
  - iii) isolating from the mixtures obtained in ii) ruthenium-bound substances; and
  - iv) comparing the said substances obtained in iii) for said sample with the substances obtained in step iii) for said control sample;
- whereby a difference between the substances obtained in said sample and said control sample indicates a possible disorder in  $\text{Ca}^{2+}$ -binding protein in said patient.
34. (Previously Presented) Use of an AzRu compound of claim 1 in the preparation of a medicament for treating a disorder associated with a defect in the function of a  $\text{Ca}^{2+}$ -binding protein.

35. (Currently Amended) A pharmaceutical composition containing an AzRu compound, or a solvate thereof, prepared by a process of ~~[any one of claims]~~ claim 26 ~~[to 32]~~.
36. (Previously Presented) A pharmaceutical composition according to claim 35, further comprising a carrier, stabilizer, adjuvant, diluent, or excipient.
37. (Previously Presented) A pharmaceutical composition according to claim 35, for use as a medicament for treating or preventing a disorder associated with a defect in the function of a Ca<sup>2+</sup>-binding protein.
38. (Previously Presented) A pharmaceutical composition according to claim 37, for inhibiting the calcium-binding activity of said Ca<sup>2+</sup>-binding protein.
39. (Previously Presented) A pharmaceutical composition according to claim 35, for use as a medicament for inhibiting apoptotic or necrotic cell death.